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Protein Kinase D1 Correlates with Less Lymph Node Metastasis Risk, Enhanced 5-FU Sensitivity, and Better Prognosis in Colorectal Cancer

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Protein kinase D1 (PKD1) controls tumor growth and invasion of gastrointestinal tract-related cancers, but its prognostic role in colorectal cancer (CRC) is not clear yet. Therefore, this research intended to assess the potential of PKD1 as a marker for CRC patients' management, also to evaluate its effect on 5-fluorouracil (5-FU) chemosensitivity in CRC cell lines. PKD1 protein and mRNA expressions were measured by immunohistochemistry and reverse transcription-quantitative polymerase chain reaction assays in 214 CRC patients, respectively. The PKD1 overexpression plasmids and negative control (NC) plasmids were transfected into the HCT-116 and LoVo cell lines followed by 0-16 μM 5-FU treatment. PKD1 protein ($P < 0.001$) and mRNA expressions ($P < 0.001$) were both descended in tumor tissues compared to tumor-adjacent tissues. Meanwhile, tumor PKD1 protein and mRNA expressions were both negatively related to lymph node metastasis, N stage, and tumor-node-metastasis (TNM) stage (all $P < 0.05$). Prognostically, high expressions of PKD1 protein and mRNA were linked with prolonged disease-free survival (DFS) and overall survival (OS) (all $P < 0.05$). After adjustment by multivariate Cox analyses, PKD1 mRNA high expression independently forecasted longer DFS [hazard ratio (HR) = 0.199, $P = 0.002$] and OS (HR = 0.212, $P = 0.022$). *In vitro* experiments revealed that PKD1 overexpression decreased the half maximal inhibitory concentration value of 5-FU in the HCT-116 ($P = 0.016$) and LoVo ($P = 0.007$) cell lines. PKD1 expression links with less lymph node metastasis risk and satisfied prognosis in CRC patients, which promotes CRC cell chemosensitivity to 5-FU chemosensitivity as well.

Keywords: 5-fluorouracil sensitivity; colorectal cancer; prognosis; protein kinase D1; tumor features
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Introduction

Colorectal cancer (CRC) is a frequent gastrointestinal tract-related cancer around the world with an approximated number of 1,850,000 new cases and 850,000 deaths each year (Baidoun et al. 2021; Biller and Schrag 2021). At present, the mainstay treatments for CRC contain surgical techniques, chemotherapy, immunotherapy, and radiotherapy (Fan et al. 2021; Shinji et al. 2022). Among them, 5-fluorouracil (5-FU) based chemotherapy has become one of the standard treatments for CRC (Blondy et al. 2020; Olguin et al. 2023). However, over 80% of patients still do not respond to the monotherapy treatment of 5-FU, and about 50-60% of patients do not respond well to the treatment of 5-FU combined with agents such as oxaliplatin and irinotecan, leading to an unsatisfactory prognosis of CRC patients (Longley et al. 2003; Allen and Johnston 2005a, b; Wong et al. 2008; Karthika et al. 2022; Pouya et al. 2022; Fekete and Gyorffy 2023). Therefore, it is crucial to search for potential biomarkers for forecasting the efficacy of 5-FU-based chemotherapy regimens and improving the stratified management of CRC.

Protein kinase D1 (PKD1) controls cell proliferation, motility, apoptosis, and other cellular functions, playing a crucial part in the progression of gastrointestinal tract-related cancers by regulating a series of signing pathways (Kim et al. 2008; Shabelnik et al. 2011; Sundram et al. 2011, 2014). For example, one study suggests that PKD1

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inhibits the progression of colon cancer by regulating *β*-catenin/T cell factor activity in colon cancer tissues (Sundram et al. 2014). Meanwhile, another study reveals that the overexpression of PKD1 suppresses cell proliferation and migration in human gastric adenocarcinoma cells (Shabelnik et al. 2011). In addition, one study discloses that the inactivation of PKD1 promotes gastric cancer cell migration and invasion (Kim et al. 2008). However, there is still a lack of relevant research that reveals the potential of PKD1 as a biomarker for the management of CRC clinically.

Therefore, the present study intended to explore the PKD1 expression and its relationship with tumor features and survival in CRC patients, as well as to evaluate its effect on 5-FU chemosensitivity in CRC cell lines.

Methods

Patients

In this retrospective study, a total of 214 non-metastatic CRC patients who came to our hospital for surgical resection from January 2017 to December 2021 were included. The inclusion criteria were: 1) diagnosed with CRC by clinicopathologic methods; 2) age ≥18 years; 3) received surgical resection; 4) had paired formalin-fixed paraffin-embedded (FFPE) specimens of tumor and tumor-adjacent tissues; 5) with at least one follow-up information that could be assessed for survival. The exclusion criteria were: 1) with distant metastasis; 2) with a history of other primary cancers or hematologic malignancies; 3) without complete preoperative clinical characteristics; 4) pregnant or lactating women. The approval was obtained from the Ethics Committee of Hunan Cancer Hospital, approval number [2021-KY61]. Informed consents were gained from all patients or their guardians.

Collection of data

Clinical features of CRC patients containing demographics and disease characteristics were screened from the electronic medical records system. Tumor-node-metastasis (TNM) stage was evaluated based on a previous guideline (Weiser 2018). Besides, adjuvant chemotherapy-related information was collected, in which 5-FU based adjuvant chemotherapy was used for patients with TNM stage II (medium or high-risk) and TNM stage III. The regimens included capecitabine alone, capecitabine combined with oxaliplatin (CapeOX), leucovorin combined with 5-FU (sLV5FU2), and oxaliplatin combined with 5-FU and leucovorin (mFOLFOX6). In this study, five patients with TNM stage II did not receive adjuvant chemotherapy. The reasons were as follows: (1) 4 patients with microsatellite instability-high, indicating that they had a good prognosis and therefore did not receive adjuvant chemotherapy. (2) The remaining 1 patient was older and personally not inclined to receive adjuvant chemotherapy. Additionally, this patient's surgical margin result was good and the degree of tumor differentiation was low. Based on comprehensive evaluation and patient's willingness, this patient did not receive adjuvant chemotherapy. Additionally, the follow-up data were obtained, and the last follow-up date was October 2022 (median follow-up time, 31.2 months; range, 6.9-66.2 months). The disease-free survival (DFS) and overall survival (OS) rates were computed.

Collection of specimens

Totally, 214 paired FFPE specimens of tumor and tumor-adjacent tissues from CRC patients were gained after surgical resection for PKD1 protein expression detection by immunohistochemistry (IHC) assay. A total of 100 available paired tumor and tumor-adjacent tissue specimens stored in liquid nitrogen were also sorted out for PKD1 mRNA expression detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

IHC assay

The experimental procedure briefly contained sample preparation, antigen recovery, block, detection, and visualization. The rabbit polyclonal to PKD1 (Abbexa, Wuhan, Hubei, China) was applied as the primary antibody at 1:200 dilution, and the goat-anti-rabbit immunoglobulin G antibody (No. Cat. abx400049, Abbexa, AmyJet Scientific Inc., Wuhan, Hubei, China) was applied as a secondary antibody at 1:100 dilution. After IHC staining, the IHC score was figured out by multiplying the intensity and density of the staining. The intensity of staining was rated as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The density of staining was scored as 0 (0%), 1 (1~25%), 2 (26~50%), 3 (51~75%), and 4 (> 75%) (Ye et al. 2020). The PKD1 protein expression was classified into high and low through value 3 of the tumor tissues' IHC score. The IHC score was evaluated by two independent pathologists, and the final score was averaged between the two IHC scores assessed by each pathologist.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted via PureZOL RNA isolation reagent (Bio-Rad, Hercules, CA, USA). cDNA was synthesized using a ReverTra Ace® qPCR RT Kit (Toyobo, Osaka, Japan). Subsequently, qPCR was conducted via KOD SYBR® qPCR Mix (Toyobo). Relative quantification was computed via the $2^{-\Delta\Delta CT}$ method. GAPDH was used as a reference. The primer of human PKD1 was as follows: forward, 5′-TCGCTCACAATGAAGTCAGC-3′ and reverse, 5′-CTGGTTCATACGGCTCCACT-3′ (Burtey et al. 2005). The PKD1 mRNA expression was classified into high and low by the median value of tumor tissues.

Cell viability

The chemosensitivity of CRC cells to 5-FU was confirmed by cell viability analysis. The pEX2 vector (Genepharma, Suzhou, China) was applied to construct negative control (NC) and PKD1 overexpression plasmids.

Then, the plasmids were transfected into HCT-116 (Procell, Wuhan, Hubei, China) and LoVo cells (Procell) via Lipofectamine™ 3000 Transfection Reagent (Invitrogen, Waltham, MA, USA), in which non-transfected cells served as control. After 48 hours, all cells were cultured with 5-FU (Selleckchem, Houston, TX, USA) for another 24 hours at concentrations of 0, 1, 2, 4, 8, and 16 μM. An initial screening of the 5-FU concentration range was first performed by referring to the previous literatures (Wang et al. 2010; Feng et al. 2017; Liu et al. 2018; Chen et al. 2020; Duarte et al. 2022; Pereira and Vale 2022). Then, pre-experiments were conducted to further narrow the concentration range. Finally, the concentration gradient in this study was determined by combing pre-experiments results with the laboratory conditions. Then, 10 μL Cell Counting Kit-8 (Topscience, Shanghai, China) was cultured with cells for 2 hours. After that, the absorbance was measured at 450 nm to determine the cell viability with a microplate reader (BioTek, Winooski, VT, USA). Finally, relative cell viability and half maximal inhibitory concentration (IC_{50}) were computed. The cell experiments were carried out in triplicate.

Statistics

The Wilcoxon signed-rank test was used for comparing PKD1 expression between different tissues, while the Mann-Whitney U test was performed for the comparison of PKD1 expression between patients with different characteristics. The spearman test was applied for correlation analysis. Kaplan-Meier curves were used to exhibit the DFS/OS between high and low expression of PKD1, in which the Log-rank test was applied. Univariate and forward-multivariate cox proportional hazards models were applied for analyzing factors associated with DFS or OS. The difference in relative cell viability or IC_{50} value between PKD1-pEX2 and NC-pEX2 groups was analyzed via a *t*-test. A $P < 0.05$ indicated significance. SPSS v.26.0 (IBM, Armonk, NY, USA) and GraphPad Prism v.7.01 (GraphPad Software, Boston, MA, USA) were utilized for the data processing and figure construction.

Results

Baseline characteristics of CRC patients

The mean age of enrolled CRC patients was 63.0 ± 10.8 years [mean ± standard deviation (SD)]. Regarding sex, CRC patients included 85 (39.7%) females and 129 (60.3%) males. Meanwhile, the mean value of tumor size was 4.4 ± 1.4 cm in CRC patients. Moreover, there were 84 (39.4%) patients with lymph node (LYN) positive. Notably, there were 28 (13.1%) patients with TNM stage I, 101 (47.2%) patients with TNM stage II, and 85 (39.7%) patients with TNM stage III. Among all participants, 181 (84.6%) patients received adjuvant chemotherapy, while 33 (15.4%) patients did not. More specific information regarding the baseline characteristics of CRC patients was shown in Table 1.

Table 1. Clinical characteristics of CRC patients.

Characteristics	CRC patients (N = 214)
Age (years), mean ± SD	63.0 ± 10.8
Sex, No. (%)	
Female	85 (39.7)
Male	129 (60.3)
ECOG PS score, No. (%)	
0	143 (66.8)
1	71 (33.2)
Differentiation, No. (%)	
Well	31 (14.5)
Moderate	150 (70.1)
Poor	33 (15.4)
MSI status, No. (%)	
MSI-H	21 (9.8)
MSI-L/MSS	193 (90.2)
Tumor size (cm), mean ± SD	4.4 ± 1.4
LYN positive, No. (%)	84 (39.4)
T stage, No. (%)	
1	5 (2.3)
2	23 (10.7)
3	183 (85.5)
4	3 (1.4)
N stage, No. (%)	
0	130 (60.6)
1	58 (27.2)
2	26 (12.2)
M stage, No. (%)	
0	214 (100.0)
TNM stage, No. (%)	
I	28 (13.1)
II	101 (47.2)
III	85 (39.7)
Adjuvant chemotherapy, No. (%)	
No	33 (15.4)
Yes	181 (84.6)

CRC, colorectal cancer; SD, standard deviation; ECOG PS, the eastern cooperative oncology group performance status; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite-stable; LYN, lymph node; TNM, tumor-node-metastasis.

Comparison of PKD1 expression between tumor and tumor-adjacent tissues in CRC patients

PKD1 protein expression was reduced in tumor tissues [median (interquartile range, IQR): 2.0 (1.0-4.0)] compared with tumor-adjacent tissues [median (IQR): 5.0 (3.0-8.0)] in CRC patients ($P < 0.001$) (Fig. 1A). Furthermore, PKD1 mRNA expression was also decreased in tumor tissues [median (IQR): 0.435 (0.300-0.923)] compared with tumor-adjacent tissues [median (IQR): 1.000 (0.723-1.528)] in CRC patients ($P < 0.001$) (Fig. 1B).

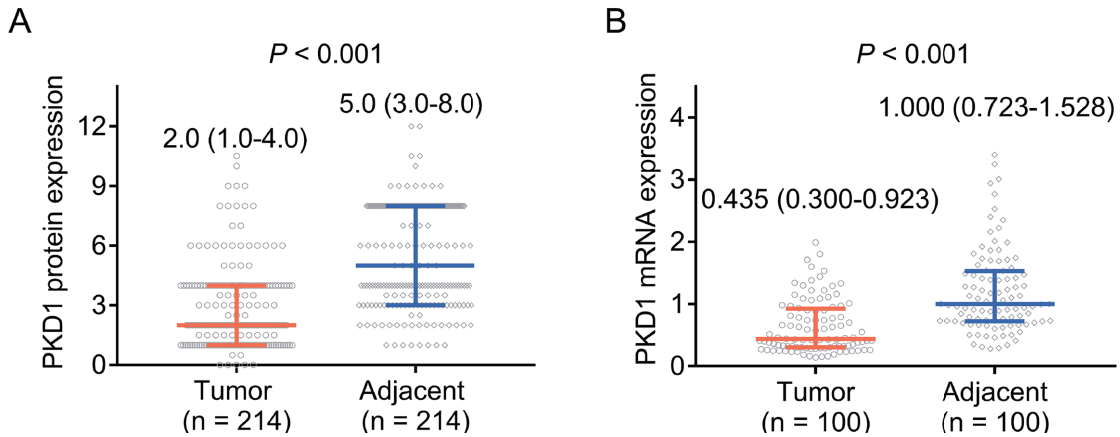


Fig. 1. Protein kinase D1 (PKD1) expression in tumor and tumor-adjacent tissues in colorectal cancer (CRC) patients. Comparison of PKD1 protein (A) and mRNA (B) expressions between tumor and tumor-adjacent tissues in CRC patients. Data are shown as median (interquartile range, IQR).

Relationship of PKD1 expression in tumor tissues with clinical properties in CRC patients

CRC patients with elevated PKD1 protein expression exhibited less LYN metastasis ($P = 0.032$), lower T stage ($P = 0.039$), lower N stage ($P = 0.045$), and lower TNM stage ($P = 0.012$) (Table 2). Furthermore, CRC patients with increased PKD1 mRNA expression also indicated less LYN metastasis ($P = 0.002$), lower N stage ($P = 0.009$), and lower TNM stage ($P = 0.001$) (Table 3).

Relationship of PKD1 expression in tumor tissues with DFS and OS in CRC patients

CRC patients with low PKD1 protein expression presented reduced DFS ($P = 0.013$) and OS ($P = 0.046$) versus CRC patients with high PKD1 protein expression (Fig. 2A, B). Simultaneously, CRC patients with low PKD1 mRNA expression exhibited declined DFS ($P = 0.005$) and OS ($P = 0.035$) in comparison with CRC patients with high PKD1 mRNA expression (Fig. 2C, D).

Factors related to DFS in CRC patients

A univariate cox proportional hazards model disclosed that PKD1 protein expression (high vs. low) [hazard ratio (HR) = 0.423, $P = 0.016$] and PKD1 mRNA expression (high vs. low) (HR = 0.273, $P = 0.008$) showed a correlation with longer DFS in CRC patients. However, poorer differentiation (HR = 3.180, $P < 0.001$), LYN positive (yes vs. no) (HR = 2.715, $P = 0.001$), higher T stage (HR = 2.367, $P = 0.039$), higher N stage (HR = 1.767, $P = 0.001$), higher TNM stage (HR = 2.681, $P < 0.001$), and adjuvant chemotherapy (yes vs. no) (HR = 4.866, $P = 0.029$) exhibited a linkage with poorer DFS in CRC patients. Next, the multivariate cox proportional hazards model exhibited that PKD1 mRNA expression (high vs. low) independently forecasted longer DFS (HR = 0.199, $P = 0.002$), but the eastern cooperative oncology group performance status score (1 vs. 0) (HR = 3.079, $P = 0.013$) and poorer differentiation (HR = 5.977, $P < 0.001$) independently forecasted worse DFS in CRC patients (Table 4).

Factors related to OS in CRC patients

A univariate cox proportional hazards model indicated that PKD1 protein expression (high vs. low) (HR = 0.385, $P = 0.054$) and PKD1 mRNA expression (high vs. low) (HR = 0.270, $P = 0.050$) tended to correlate with longer OS in CRC patients, but they did not achieve statistical significance. In addition, poorer differentiation (HR = 2.269, $P = 0.020$), tumor size (≥ 5 cm vs. < 5 cm) (HR = 2.698, $P = 0.011$), LYN positive (yes vs. no) (HR = 3.115, $P = 0.004$), higher N stage (HR = 1.706, $P = 0.021$), and higher TNM stage (HR = 2.983, $P = 0.002$) were correlated with poorer OS in CRC patients. Next, the multivariate cox proportional hazards model disclosed that PKD1 mRNA expression (high vs. low) independently forecasted longer OS (HR = 0.212, $P = 0.022$), while poorer differentiation independently forecasted worse OS (HR = 5.088, $P = 0.001$) in CRC patients (Table 5). Notably, PKD1 protein expression (high vs. low) was not linked with DFS (HR = 0.009, $P = 0.437$) or OS (HR = 0.012, $P = 0.594$) in CRC patients who did not receive adjuvant chemotherapy.

Effect of PKD1 on 5-FU chemosensitivity in CRC cell lines

In the HCT-116 cell line, relative cell viability was descended in PKD1-overexpressed cells (with PKD1-pEX2) versus NC (with NC-pEX2) under 2 ($P = 0.015$), 4 ($P = 0.024$), 8 ($P = 0.007$), and 16 ($P = 0.049$) μM 5-FU treatment, respectively (Fig. 3A). Moreover, the IC_{50} value of 5-FU was reduced in PKD1-overexpressed cells compared with NC (4.5 ± 0.4 vs. 9.4 ± 2.1 μM) ($P = 0.016$) (Fig. 3B). Similarly, in the LoVo cell line, relative cell viability was declined in PKD1-overexpressed cells versus NC under 2 ($P = 0.045$) and 8 ($P = 0.026$) μM 5-FU (Fig. 3C). Notably, the IC_{50} value of 5-FU was descended in PKD1-overexpressed cells compared with NC (4.9 ± 1.0 vs. 7.9 ± 0.2 μM) ($P = 0.007$) (Fig. 3D).

Table 2. The correlation of PKD1 protein expression in tumor tissues with clinical characteristics.

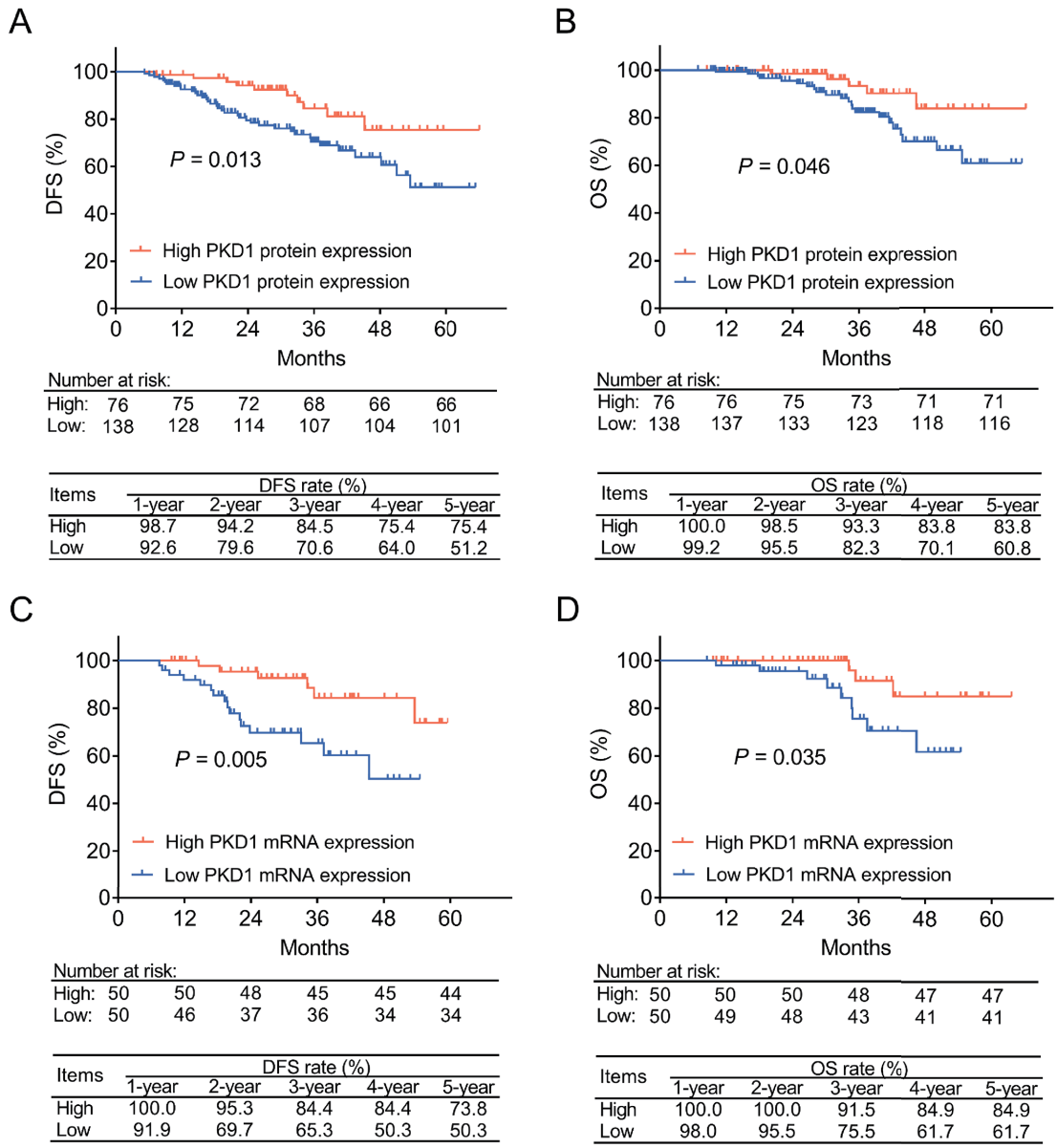
Characteristics	PKD1 protein expression, median (IQR)	*P* value
Age		0.189
< 60 years	2.0 (1.5-4.0)	
≥ 60 years	2.0 (1.0-4.0)	
Sex		0.211
Female	2.0 (1.5-4.0)	
Male	2.0 (1.0-4.0)	
ECOG PS score		0.158
0	2.0 (1.0-4.0)	
1	2.0 (1.0-4.0)	
Differentiation		0.296
Well	2.0 (1.5-4.0)	
Moderate	2.0 (1.0-4.0)	
Poor	2.0 (1.0-3.0)	
MSI status		0.151
MSI-H	4.0 (1.3-5.0)	
MSI-L/MSS	2.0 (1.0-4.0)	
Tumor size		0.587
< 5 cm	2.0 (1.0-4.0)	
≥ 5 cm	2.0 (1.0-4.0)	
LYN positive		0.032
No	2.0 (1.5-4.0)	
Yes	2.0 (1.0-3.9)	
T stage		0.039
1	4.0 (2.0-7.5)	
2	2.0 (2.0-4.0)	
3	2.0 (1.0-4.0)	
4	1.5 (1.0-NA)	
N stage		0.045
0	2.0 (1.5-4.0)	
1	2.0 (1.0-3.6)	
2	2.0 (1.0-4.0)	
TNM stage		0.012
I	2.8 (2.0-4.0)	
II	2.0 (1.0-4.0)	
III	2.0 (1.0-3.8)	
Adjuvant chemotherapy		0.122
No	2.0 (1.8-4.0)	
Yes	2.0 (1.0-4.0)	

PKD1, protein kinase D1; IQR, interquartile range; ECOG PS, the eastern cooperative oncology group performance status; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite-stable; LYN, lymph node; NA, not available; TNM, tumor-node-metastasis.
The PKD1 protein expression was in skewed distribution, which was determined by the Kolmogorov-Smirnov test.

Table 3. The correlation of PKD1 mRNA expression in tumor tissues with clinical characteristics.

Characteristics	PKD1 mRNA expression, median (IQR)	*P* value
Age		0.822
< 60 years	0.430 (0.320-0.785)	
≥ 60 years	0.440 (0.280-0.970)	
Sex		0.366
Female	0.600 (0.295-0.975)	
Male	0.430 (0.300-0.900)	
ECOG PS score		0.209
0	0.460 (0.320-0.950)	
1	0.400 (0.280-0.835)	
Differentiation		0.454
Well	0.430 (0.285-1.065)	
Moderate	0.460 (0.315-0.893)	
Poor	0.360 (0.270-0.845)	
MSI status		0.758
MSI-H	0.430 (0.290-1.220)	
MSI-L/MSS	0.440 (0.300-0.900)	
Tumor size		0.575
< 5 cm	0.485 (0.300-0.965)	
≥ 5 cm	0.415 (0.293-0.878)	
LYN positive		0.002
No	0.560 (0.328-1.120)	
Yes	0.380 (0.258-0.673)	
T stage		0.335
2	0.955 (0.385-1.285)	
3	0.425 (0.298-0.893)	
4	0.760 (0.740-NA)	
N stage		0.009
0	0.560 (0.328-1.120)	
1	0.335 (0.248-0.635)	
2	0.580 (0.410-0.920)	
TNM stage		0.001
I	0.955 (0.385-1.285)	
II	0.485 (0.328-1.095)	
III	0.380 (0.258-0.673)	
Adjuvant chemotherapy		0.162
No	0.930 (0.320-1.220)	
Yes	0.430 (0.295-0.850)	

PKD1, protein kinase D1; mRNA, messenger ribonucleic acid; IQR, interquartile range; ECOG PS, the eastern cooperative oncology group performance status; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite-stable; LYN, lymph node; NA, not available; TNM, tumor-node-metastasis.
The PKD1 mRNA expression was in skewed distribution, which was determined by the Kolmogorov-Smirnov test.



Items	DFS rate (%)				
	1-year	2-year	3-year	4-year	5-year
High	98.7	94.2	84.5	75.4	75.4
Low	92.6	79.6	70.6	64.0	51.2

Items	OS rate (%)				
	1-year	2-year	3-year	4-year	5-year
High	100.0	98.5	93.3	83.8	83.8
Low	99.2	95.5	82.3	70.1	60.8

Items	DFS rate (%)				
	1-year	2-year	3-year	4-year	5-year
High	100.0	95.3	84.4	84.4	73.8
Low	91.9	69.7	65.3	50.3	50.3

Items	OS rate (%)				
	1-year	2-year	3-year	4-year	5-year
High	100.0	100.0	91.5	84.9	84.9
Low	98.0	95.5	75.5	61.7	61.7

Fig. 2. Association of protein kinase D1 (PKD1) expression with disease-free survival (DFS) and overall survival (OS) in colorectal cancer (CRC) patients.
The association of PKD1 protein expression with DFS (A) and OS (B). The association of PKD1 mRNA expression with DFS (C) and OS (D) in CRC patients.

Discussion

As the most widely studied PKD subtype, PKD1 has been proven to be dysregulated in many gastrointestinal tract-related cancers (Kim et al. 2008; Sundram et al. 2014; Zhang et al. 2021). For instance, one study indicates that PKD1 is reduced in gastric tumor tissue compared to normal tissue (Kim et al. 2008). Meanwhile, another study discloses that PKD1 is descended in colon cancer tissues versus normal tissues (Sundram et al. 2014). Similar to these studies, our study revealed that the PKD1 protein and mRNA expressions were descended in tumor tissues versus tumor-adjacent tissues in CRC patients. We hypothesized that the reduction of PKD1 expression in CRC might be due to the cytosine-phosphate-guanine island hypermethylation in the PKD1 promoter (Kim et al. 2008). Therefore, PKD1 expression was decreased in tumor tissues in CRC patients. Meanwhile, our study showed that PKD1 protein and mRNA expressions in tumor tissues were inversely correlated with LYN metastasis, N stage, and TNM stage. These findings might be because: (1) PKD1 phosphorylated metastasis-associated protein 1, which inhibited CRC cell migration (Ganju et al. 2018). Therefore, PKD1 expression in tumor tissues was negatively linked with LYN metastasis and N stage in CRC patients. (2) PKD1 inhibited CRC cell proliferation by restraining the transcriptional activity of

Table 4. Cox proportional hazards models for DFS.

Items	*P* value	HR	95% CI	
			Lower	Upper
Univariate cox proportional hazards model				
PKD1 protein expression, high vs. low	0.016	0.423	0.210	0.852
PKD1 mRNA expression, high vs. low	0.008	0.273	0.105	0.714
Age, ≥ 60 years vs. < 60 years	0.430	1.268	0.703	2.284
Sex, male vs. female	0.404	1.294	0.706	2.372
ECOG PS score, 1 vs. 0	0.135	1.570	0.870	2.833
Poorer differentiation	< 0.001	3.180	1.862	5.431
MSI status, MSI-H vs. MSI-L/MSS	0.113	0.041	0.001	2.124
Tumor size, ≥ 5 cm vs. < 5 cm	0.593	1.174	0.652	2.115
LYN positive, yes vs. no	0.001	2.715	1.513	4.870
Higher T stage	0.039	2.367	1.042	5.377
Higher N stage	0.001	1.767	1.248	2.503
Higher TNM stage	< 0.001	2.681	1.611	4.463
Adjuvant chemotherapy, yes vs. no	0.029	4.866	1.178	20.102
Multivariate cox proportional hazards model				
PKD1 mRNA expression, high vs. low	0.002	0.199	0.071	0.561
ECOG PS score, 1 vs. 0	0.013	3.079	1.273	7.446
Poorer differentiation	< 0.001	5.977	2.608	13.697

DFS, disease-free survival; HR, hazards ratio; CI, confidence interval; PKD1, protein kinase D1; mRNA, messenger ribonucleic acid; ECOG PS, the eastern cooperative oncology group performance status; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite-stable; LYN, lymph node; TNM, tumor-node-metastasis.

Table 5. Cox proportional hazards models for OS.

Items	*P* value	HR	95% CI	
			Lower	Upper
Univariate cox proportional hazards model				
PKD1 protein expression, high vs. low	0.054	0.385	0.146	1.018
PKD1 mRNA expression, high vs. low	0.050	0.270	0.073	0.999
Age, ≥ 60 years vs. < 60 years	0.628	1.209	0.560	2.609
Sex, male vs. female	0.904	1.049	0.485	2.266
ECOG PS score, 1 vs. 0	0.087	1.962	0.907	4.247
Poorer differentiation	0.020	2.269	1.135	4.536
MSI status, MSI-H vs. MSI-L/MSS	0.235	0.041	< 0.001	7.965
Tumor size, ≥ 5 cm vs. < 5 cm	0.011	2.698	1.251	5.819
LYN positive, yes vs. no	0.004	3.115	1.425	6.811
Higher T stage	0.145	2.143	0.768	5.979
Higher N stage	0.021	1.706	1.085	2.680
Higher TNM stage	0.002	2.983	1.500	5.932
Adjuvant chemotherapy, yes vs. no	0.079	6.002	0.811	44.414
Multivariate cox proportional hazards model				
PKD1 mRNA expression, high vs. low	0.022	0.212	0.056	0.797
Poorer differentiation	0.001	5.088	1.869	13.847

OS, overall survival; HR, hazards ratio; CI, confidence interval; PKD1, protein kinase D1; mRNA, messenger ribonucleic acid; ECOG PS, the eastern cooperative oncology group performance status; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite-stable; LYN, lymph node; TNM, tumor-node-metastasis.

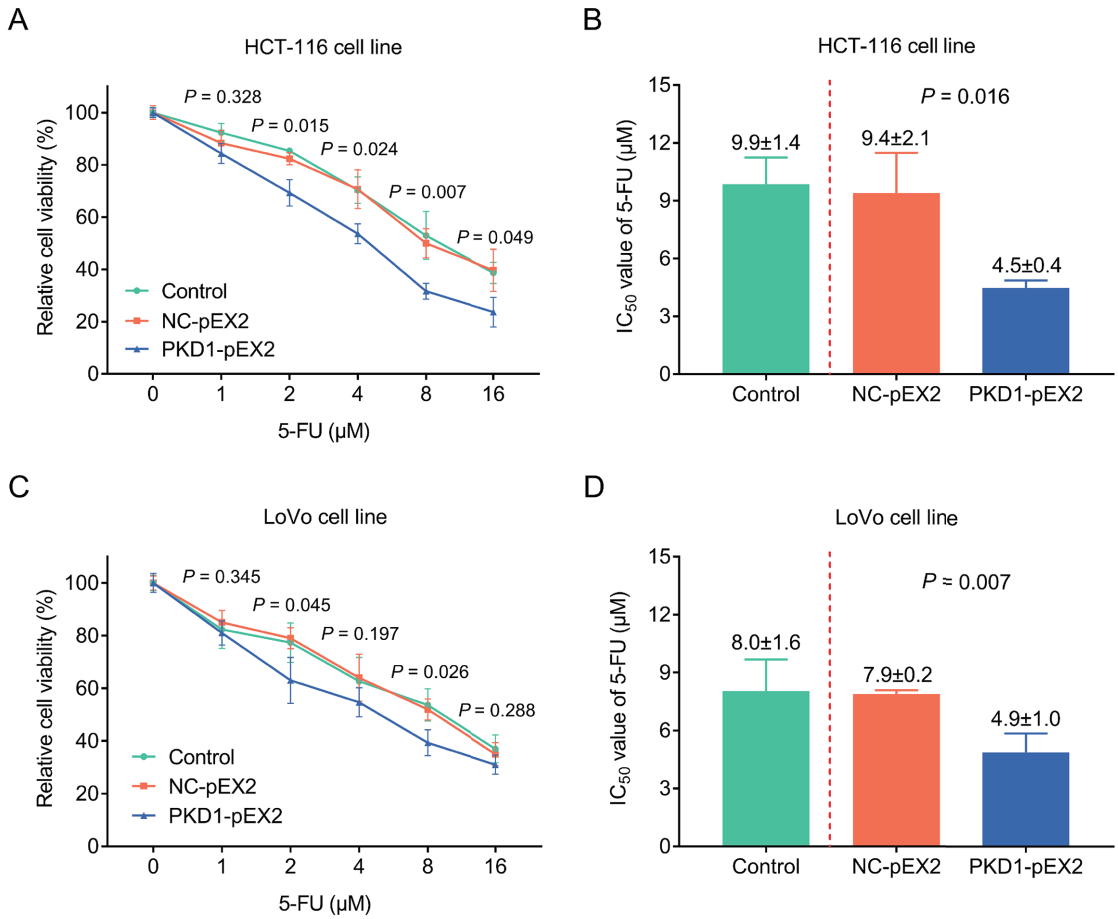


Fig. 3. Relative cell viability and IC_{50} value of 5-fluorouracil (5-FU) in colorectal cancer (CRC) cell lines. Comparison of relative cell viability (A) and IC_{50} value of 5-FU (B) between protein kinase D1 (PKD1)-overexpressed cells (with PKD1-pEX2) and negative control (NC) (with NC-pEX2) in HCT-116 cell line. Comparison of relative cell viability (C) and IC_{50} value of 5-FU (D) between PKD1-overexpressed cells (with PKD1-pEX2) and NC (with NC-pEX2) in LoVo cell line. Error bars mean standard deviation (SD). The cell experiments were performed in triplicate (n = 3).

β-catenin and inhibited CRC cell invasion by inversely regulating the expression of matrix metalloproteinases (Eiseler et al. 2009; Sundram et al. 2014). Meanwhile, as mentioned above, PKD1 also inhibited the migration of CRC cells (Ganju et al. 2018). Therefore, PKD1 was negatively associated with the TNM stage in CRC patients.

Notably, the correlation between PKD1 and survival in CRC patients has not been evaluated in previous research. In our study, PKD1 independently forecasted longer DFS and OS in CRC patients. The finding might be due to the fact that: (1) PKD1 inhibited the progression of CRC by inhibiting *β*-catenin/T cell factor activity, which resulted in lower tumor burden in CRC patients (Sundram et al. 2014). Therefore, PKD1 was associated with good survival in CRC patients. (2) PKD1 was proven to be inversely associated with higher tumor stage as mentioned above, thus it was associated with good prognosis in CRC patients. (3) PKD1 might promote the chemosensitivity of 5-FU in CRC cells, which resulted in better treatment outcomes; thus, it was associated with better prognosis of CRC patients (Huang et al. 2022).

5-FU induces cancer cell death through suppressing thymidylate synthase activity, which is one of the commonly used drugs in the treatment of CRC patients (Sethy and Kundu 2021; Huang et al. 2022; Zhao et al. 2022). However, many CRC patients do not respond to the 5-FU therapy, which negatively affects their treatment outcomes (Sethy and Kundu 2021). Therefore, it is important to seek some measures to increase 5-FU chemosensitivity. A previous study shows that elevated p21-activated protein kinase1 expression is linked with malignant progression of CRC, and the knockdown of PAK1 increases the chemotherapy sensitivity of CRC to 5-FU (Qing et al. 2012). It stood to reason that, PKD1, which was associated with less lymph node metastasis risk and satisfied prognosis in CRC patients, also might be a new treatment strategy for increasing 5-FU chemosensitivity in CRC cell lines. Our study conducted a preliminary exploration, which found that PKD1 promoted 5-FU chemosensitivity in CRC cell lines. The possible explanation was as follows: PKD1 could promote the chemosensitivity of 5-FU in CRC cell lines through some ways: such as inhibiting the *β*-catenin activity

(Sundram et al. 2014; Dong et al. 2022), and inhibiting phosphoinositide 3-kinase/protein kinase B activation to reduce the hypoxia inducible factor 1α levels (Ni et al. 2013; Dong et al. 2022). Therefore, PKD1 might increase the chemosensitivity of 5-FU. However, the specific mechanism of PKD1 promoting 5-FU chemosensitivity in CRC cell lines was unclear and needed to be further explored.

This study existed several limitations: (1) Our study only detected PKD1 at a certain point in time, and the longitudinal change of PKD1 in CRC patients should be evaluated in further studies. (2) The specific mechanism of PKD1 promoting 5-FU chemosensitivity in CRC cell lines should be analyzed in future research. (3) Our study was a single-center study, thus multiple-center studies should be needed for confirmation.

In conclusion, PKD1 is not only related to lower tumor stage, longer DFS and OS, but also facilitates 5-FU chemosensitivity. Our study suggests that PKD1 serves as a potential marker for the prognostic stratification of CRC.

Author Contributions

Zhuo He: Conceptualization; formal analysis and investigation; writing original draft preparation, review and editing; and supervision. Bo Huang: Methodology; writing original draft preparation, review and editing; and resources. All authors participated in discussions about the article and approved the final version.

Conflict of Interest

The authors declare no conflict of interest.

References

Allen, W.L. & Johnston, P.G. (2005a) Have we made progress in pharmacogenomics? The implementation of molecular markers in colon cancer. *Pharmacogenomics*, **6**, 603-614.

Allen, W.L. & Johnston, P.G. (2005b) Role of genomic markers in colorectal cancer treatment. *J. Clin. Oncol.*, **23**, 4545-4552.

Baidoun, F., Elshiwy, K., Elkeraie, Y., Merjaneh, Z., Khoudari, G., Sarmini, M.T., Gad, M., Al-Husseini, M. & Saad, A. (2021) Colorectal cancer epidemiology: recent trends and impact on outcomes. *Curr. Drug Targets*, **22**, 998-1009.

Biller, L.H. & Schrag, D. (2021) Diagnosis and treatment of metastatic colorectal cancer: a review. *JAMA*, **325**, 669-685.

Blondy, S., David, V., Verdier, M., Mathonnet, M., Perraud, A. & Christou, N. (2020) 5-fluorouracil resistance mechanisms in colorectal cancer: from classical pathways to promising processes. *Cancer Sci.*, **111**, 3142-3154.

Burtey, S., Leclerc, C., Nabais, E., Munch, P., Gohory, C., Moreau, M. & Fontes, M. (2005) Cloning and expression of the amphibian homologue of the human PKD1 gene. *Gene*, **357**, 29-36.

Chen, B., Xu, X., Zheng, K., Liu, L., Yu, Y. & Xin, Y. (2020) Konjac glucomannan reverses multi-drug resistance of HepG2/5-FU cells by suppressing AKT signaling and increasing p53 expression. *Oncol. Lett.*, **20**, 2105-2112.

Dong, S., Liang, S., Cheng, Z., Zhang, X., Luo, L., Li, L., Zhang, W., Li, S., Xu, Q., Zhong, M., Zhu, J., Zhang, G. & Hu, S. (2022) ROS/PI3K/Akt and Wnt/β-catenin signalings activate HIF-1α-induced metabolic reprogramming to impart 5-fluorouracil resistance in colorectal cancer. *J. Exp. Clin. Cancer Res.*, **41**, 15.

Duarte, D., Falcao, S.I., El Mehdi, I., Vilas-Boas, M. & Vale, N. (2022) Honeybee venom synergistically enhances the cytotoxic effect of CNS drugs in HT-29 colon and MCF-7 breast cancer cell lines. *Pharmaceutics*, **14**, 511.

Eiseler, T., Doppler, H., Yan, I.K., Goodison, S. & Storz, P. (2009) Protein kinase D1 regulates matrix metalloproteinase expression and inhibits breast cancer cell invasion. *Breast Cancer Res.*, **11**, R13.

Fan, A., Wang, B., Wang, X., Nie, Y., Fan, D., Zhao, X. & Lu, Y. (2021) Immunotherapy in colorectal cancer: current achievements and future perspective. *Int. J. Biol. Sci.*, **17**, 3837-3849.

Fekete, J.T. & Gyorffy, B. (2023) New transcriptomic biomarkers of 5-fluorouracil resistance. *Int. J. Mol. Sci.*, **24**, 1508.

Feng, X., Luo, Q., Zhang, H., Wang, H., Chen, W., Meng, G. & Chen, F. (2017) The role of NLRP3 inflammasome in 5-fluorouracil resistance of oral squamous cell carcinoma. *J. Exp. Clin. Cancer Res.*, **36**, 81.

Ganju, A., Chauhan, S.C., Hafeez, B.B., Doxtater, K., Tripathi, M.K., Zafar, N., Yallapu, M.M., Kumar, R. & Jaggi, M. (2018) Protein kinase D1 regulates subcellular localisation and metastatic function of metastasis-associated protein 1. *Br. J. Cancer*, **118**, 587-599.

Huang, X., Ke, K., Jin, W., Zhu, Q., Zhu, Q., Mei, R., Zhang, R., Yu, S., Shou, L., Sun, X., Feng, J., Duan, T., Mou, Y., Xie, T., Wu, Q., et al. (2022) Identification of genes related to 5-fluorouracil based chemotherapy for colorectal cancer. *Front. Immunol.*, **13**, 887048.

Karthika, C., Sureshkumar, R., Zehravi, M., Akter, R., Ali, F., Ramproshad, S., Mondal, B., Kundu, M.K., Dey, A., Rahman, M.H., Antonescu, A. & Cavalu, S. (2022) Multidrug resistance in cancer cells: focus on a possible strategy plan to address colon carcinoma cells. *Life* (*Basel*), **12**, 811.

Kim, M., Jang, H.R., Kim, J.H., Noh, S.M., Song, K.S., Cho, J.S., Jeong, H.Y., Norman, J.C., Caswell, P.T., Kang, G.H., Kim, S.Y., Yoo, H.S. & Kim, Y.S. (2008) Epigenetic inactivation of protein kinase D1 in gastric cancer and its role in gastric cancer cell migration and invasion. *Carcinogenesis*, **29**, 629-637.

Liu, J., Huang, Y., Wang, H. & Wu, D. (2018) MiR-106a-5p promotes 5-FU resistance and the metastasis of colorectal cancer by targeting TGFβR2. *Int. J. Clin. Exp. Pathol.*, **11**, 5622-5634.

Longley, D.B., Harkin, D.P. & Johnston, P.G. (2003) 5-fluorouracil: mechanisms of action and clinical strategies. *Nat. Rev. Cancer*, **3**, 330-338.

Ni, Y., Sinnett-Smith, J., Young, S.H. & Rozengurt, E. (2013) PKD1 mediates negative feedback of PI3K/Akt activation in response to G protein-coupled receptors. *PLoS One*, **8**, e73149.

Olguin, J.E., Mendoza-Rodriguez, M.G., Sanchez-Barrera, C.A. & Terrazas, L.I. (2023) Is the combination of immunotherapy with conventional chemotherapy the key to increase the efficacy of colorectal cancer treatment? *World J. Gastrointest. Oncol.*, **15**, 251-267.

Pereira, M. & Vale, N. (2022) Repurposing alone and in combination of the antiviral saquinavir with 5-fluorouracil in prostate and lung cancer cells. *Int. J. Mol. Sci.*, **23**, 12240.

Pouya, F.D., Gazouli, M., Rasmi, Y., Lampropoulou, D.I. & Nemati, M. (2022) MicroRNAs and drug resistance in colorectal cancer with special focus on 5-fluorouracil. *Mol. Biol. Rep.*, **49**, 5165-5178.

Qing, H., Gong, W., Che, Y., Wang, X., Peng, L., Liang, Y., Wang, W., Deng, Q., Zhang, H. & Jiang, B. (2012) PAK1-dependent MAPK pathway activation is required for colorectal cancer cell proliferation. *Tumour Biol.*, **33**, 985-994.

Sethy, C. & Kundu, C.N. (2021) 5-Fluorouracil (5-FU) resistance and the new strategy to enhance the sensitivity against cancer: implication of DNA repair inhibition. *Biomed. Pharmacother.*, **137**, 111285.

Shabelnik, M.Y., Kovalevska, L.M., Yurchenko, M.Y., Shlapatska, L.M., Rzepetsky, Y. & Sidorenko, S.P. (2011) Differential expression of PKD1 and PKD2 in gastric cancer and analysis of PKD1 and PKD2 function in the model system. *Exp. Oncol.*, **33**, 206-211.

Shinji, S., Yamada, T., Matsuda, A., Sonoda, H., Ohta, R., Iwai, T., Takeda, K., Yonaga, K., Masuda, Y. & Yoshida, H. (2022) Recent advances in the treatment of colorectal cancer: a review. *J. Nippon Med. Sch.*, **89**, 246-254.

Sundram, V., Chauhan, S.C. & Jaggi, M. (2011) Emerging roles of protein kinase D1 in cancer. *Mol. Cancer Res.*, **9**, 985-996.

Sundram, V., Ganju, A., Hughes, J.E., Khan, S., Chauhan, S.C. & Jaggi, M. (2014) Protein kinase D1 attenuates tumorigenesis in colon cancer by modulating *β*-catenin/T cell factor activity. *Oncotarget*, **5**, 6867-6884.

Wang, C.J., Stratmann, J., Zhou, Z.G. & Sun, X.F. (2010) Suppression of microRNA-31 increases sensitivity to 5-FU at an early stage, and affects cell migration and invasion in HCT-116 colon cancer cells. *BMC Cancer*, **10**, 616.

Weiser, M.R. (2018) AJCC 8th edition: colorectal cancer. *Ann. Surg. Oncol.*, **25**, 1454-1455.

Wong, C.S., Wong, V.W., Chan, C.M., Ma, B.B., Hui, E.P., Wong, M.C., Lam, M.Y., Au, T.C., Chan, W.H., Cheuk, W. & Chan, A.T. (2008) Identification of 5-fluorouracil response proteins in colorectal carcinoma cell line SW480 by two-dimensional electrophoresis and MALDI-TOF mass spectrometry. *Oncol. Rep.*, **20**, 89-98.

Ye, Q., Zhang, M. & Yin, Y. (2020) Katanin P80 correlates with larger tumor size, lymph node metastasis, and advanced TNM stage and predicts poor prognosis in non-small-cell lung cancer patients. *J. Clin. Lab. Anal.*, **34**, e23141.

Zhang, X., Connelly, J., Chao, Y. & Wang, Q.J. (2021) Multifaceted functions of protein kinase D in pathological processes and human diseases. *Biomolecules*, **11**, 483.

Zhao, Q., Long, Y., Cheng, W., Huang, Y., Li, J., Li, Y., Li, X., Guo, X., Li, Y., Li, G., Gong, K. & Zhang, J. (2022) Visfatin inhibits colon cancer cell apoptosis and decreases chemosensitivity to 5-FU by promoting the SDF-1/CXCR4/Akt axis. *Int. J. Oncol.*, **60**, 75.